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A Randomized Trial of Amifostine and Carmustine-Containing Chemotherapy to Assess Lung-Protective Effects

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ABSTRACT

We conducted a randomized, double blind, placebo-controlled multi-institutional trial to assess the ability of amifostine to protect patients against acute lung injury associated with cyclophosphamide/cisplatin/carmustine (BCNU) (STAMP I), a BCNU-containing high dose chemotherapy regimen used with hematopoietic cell transplantation. Amifostine was administered in a dose of 740 mg/m^2 for 2 doses preceding administration of BCNU, the presumed pulmonary-toxic component of the regimen. The trial was stopped after 79 patients were randomized and a planned interim analysis demonstrated that it was unlikely that pulmonary cytoprotection would be detected with further accrual. We conclude that amifostine, used in the dose and schedule we tested, does not reduce the incidence of acute lung injury produced by STAMP I. Further, we suggest that amifostine use with BCNU in other contexts and with clinically achievable doses is unlikely to protect the lung from BCNU-associated acute injury.

KEY WORDS

Amifostine • Acute lung injury • Hematopoietic cell transplantation • BCNU

INTRODUCTION

Amifostine (Ethyol, Medimmune, Gaithersburg, MD; WR-2721) is a phosphorylated sulfhydryl analog of glutathione, the major intracellular protectant against electrophilic reactive intermediates [1]. Its Food and Drug Administration–approved indication is protection against cisplatin-induced renal tubular injury [2]. Additionally, randomized controlled trials have provided evidence for protection against radiation-induced xerostomia and mucositis [3], cisplatin-induced peripheral neuropathy [2], and neutropenia from cyclophosphamide [2]. There are also clinical phase II data supporting protection against melphalan-associated mucositis [4], ototoxicity from cisplatin [5], and esophagitis from radiation [6], as well as other tissue-protective effects. Both preclinical and clinical data provide strong evidence that these effects are produced without tumor protection in vivo [7,8]. Finally, preclinical data suggest that amifostine can react with electrophilic intermediates of most alkylating agents and, perhaps, with reactive intermediates produced by other classes of antineoplastic agents such as anthracyclines [7]. Taken together, available data support the conclusion that amifostine can produce clinically important cytoprotection without compromising the antitumor effect during a variety of chemotherapy and radiation treatment programs.

Carmustine (BCNU) is an alkylating agent that is used extensively in high-dose chemotherapy regimens given with hematopoietic cells. In high doses, BCNU produces acute lung injury that is steroid responsive but that can be fatal, particularly if diagnosis is delayed [9,10]. The incidence of this lung injury varies widely between regimens, with a previously reported approx-

imate range of 5% to 55% [11-13]. BCNU continues to be used, however, because of its antitumor potency and lack of gastrointestinal mucosal toxicity, a frequent side effect of hematopoietic transplant regimens. Thus, this toxicity is clinically important for patients undergoing hematopoietic cell transplantation.

We hypothesized that the use of amifostine with an intensive BCNU-containing chemotherapy regimen would reduce the incidence of BCNU-associated acute lung injury seen with the regimen. For this purpose, we used the regimen of cyclophosphamide/cisplatin/BCNU (STAMP I), which contains a large dose (600 mg/m^2) of BCNU. This regimen is well described to produce acute lung injury in 30% to 55% of patients treated [10]. To test the hypothesis, we conducted a randomized, double-blind, placebo-controlled trial at 3 institutions (University of Colorado, Stanford University, and Karmanos Cancer Center/Wayne State University) familiar with the STAMP I regimen and diagnosis of the acute lung injury it produces.

PATIENTS AND METHODS

Eligibility and Treatment

Patients eligible for this trial had a biopsy-proven diagnosis of stage II to IV breast cancer and were treated with the STAMP I regimen. They received cyclophosphamide 1875 mg/m^2/d for 3 consecutive days as a 1-hour infusion, cisplatin 55 mg/m^2/d for 3 consecutive days as a continuous infusion, and BCNU 600 mg/m^2 as a 2-hour infusion administered immediately after completion of the cisplatin. All drugs were administered through a central venous catheter. If BCNU or amifostine resulted in clinically relevant hypotension, a saline infusion and dopamine were used to augment blood pressure. Antiemetic support was that routinely used by the individual transplant programs.

Additional eligibility criteria included acceptable hepatic, renal, and cardiac function. In the case of pulmonary function, all patients were required to have a diffusing capacity of the lung for carbon monoxide (DLCO) and forced expiratory volume in 1 second $\geq$60% of the predicted value. Other eligibility criteria were those dictated by the chemotherapy protocol itself and have been previously described [14]. This study was approved and monitored by the Colorado Multiple Institutional Review Board and the Stanford University and Karmanos Institutional Review Boards. All patients gave and signed written informed consent before participation in this study.

Amifostine Administration

Amifostine 740 mg/m^2 per dose or placebo was administered as two 5-min infusions beginning 90 and 30 minutes, respectively, before the BCNU infusion was started. Amifostine was infused through a lumen of the central venous catheter separate from the chemotherapy. Amifostine was prepared by each hospital's inpatient pharmacy, and equivalent intravenous fluid without amifostine was used as placebo. Physicians, physician extenders, and nursing and data management staff were blinded to the identity of the administered agent. Although provision to unblind the staff in case of a significant adverse event was present, it was never used. Alza Pharmaceuticals (Palo Alto, CA) provided the amifostine used in this study.

Patient Evaluation and Trial Design

This trial was designed as a 3-phase study, with interim evaluations between phases. The objective of the first (feasibility) phase was to treat 3 patients at each institution with STAMP I plus amifostine in the exact dose and schedule proposed for the trial and to confirm that the regimen was tolerable. This phase was completed at the collaborating institutions, and no unanticipated or intolerable adverse effects related to amifostine were noted. During the second (randomized treatment) phase, 80 patients were to be accrued, and their treatment was to be randomized to either amifostine or placebo. An interim evaluation of outcome was prospectively planned and performed after all patients had been followed up for at least 6 months from treatment. If the interim evaluation had been positive, a third (efficacy) phase was planned with enrollment of an additional 80 patients. In fact, the trial was stopped after the second phase in view of the statistical likelihood that additional accrual would not lead to a positive result, as discussed below.

Definition of Acute Lung Injury

For the purposes of this study, acute lung injury was suspected whenever a study subject developed new dyspnea, either at rest or exercise, or when abnormal pulmonary function testing was noted without symptoms. If the patient was symptomatic, exercise pulse oximetry or pulmonary function testing was performed. Either of 2 findings would indicate possible acute lung injury, even if these tests were performed routinely without symptoms: (1) a 25% decrease in DLCO when compared with either the pre–bone marrow transplant or discharge DLCO, whichever was the more recent, or (2) a $>$7% decrease in blood oxygen saturation with maximal exercise, as measured by pulse oximetry.

Further, confounding factors that might produce dyspnea, such as infection, poor cardiac function, clinically evident embolism, and severe anemia, must be absent. Patients who developed possible acute lung injury while neutropenic were required to undergo bronchoscopy or lung biopsy to eliminate infectious

causes of pulmonary abnormality. Thus, patients identified as having possible pulmonary gas exchange defects in the absence of signs of infection or circulatory dysfunctions were eligible for enrollment in this study. Histologic proof of acute lung injury was not required for study entry.

It was recommended that all these patients receive empiric corticosteroid therapy. At the time of acute lung injury, many of these patients had returned home from the transplant center, and thus the pattern of treatment varied widely. As a result, no attempt was made to evaluate treatment outcome in these patients.

Accrual and Statistical Methods

After the initial 9 patients were accrued to the phase I feasibility pilot study (these patients were not included in the efficacy analysis), 79 patients were accrued, and in 78 their administered treatment was randomized to amifostine or placebo. An 80th patient was not accrued to avoid confusion between 2 collaborating centers and inadvertent overenrollment. One patient was enrolled, but chemotherapy complications before the administration of BCNU and amifostine caused the patient not to be treated with the study drug. This patient was not included in the analysis. Registration and randomization were performed by using a secure Internet site with password protection. Follow-up data on the occurrence of acute lung injury, duration of observation, and survival were collected in the same manner.

The initial planned phase II and III accrual of 160 patients was determined assuming a 50% relative reduction in the incidence of acute lung injury from amifostine. The assumed baseline incidence of acute lung injury from STAMP I was in the range of 30% to 55%, so a baseline rate of 40% was used for statistical purposes. We likewise assumed that failure to reduce the incidence to <20% was not a clinically meaningful result. On the basis of a 1-tailed significance level of $P = .05$ and a power of 0.8, the proposed sample size should have provided a detectable benefit from amifostine with the Fisher exact test.

The interim analysis after phase II was completed, and all patients were observed for at least 6 months. At this point, the data were examined for the incidence of acute lung injury. A total of 75% of the amifostine group and 66% of the placebo group had developed acute lung injury within this period. A total of 85% of the amifostine group and 89% of the placebo group were alive at this time. In both cases, benefit seemed to accrue to the placebo group, although neither of these differences was statistically significant. There was no significant difference in the average age at which treatment began: 38.6 years for amifostine and 40.45 years for placebo.

Table 1. *Patient Characteristics by Treatment Center*

Variable	Colorado	Stanford	Karmanos	Total
n	34	32	12	78
Age, y, mean (SD)	45 (9)	46 (8)	46 (6)	46 (8)
Stage II, n (%)	32 (94)	28 (88)	10 (83)	70 (90)
Interval,* d (SD)				
Amifostine	78 (62)	52 (9)	66 (44)	63 (40)
Placebo	77 (60)	58 (35)	34 (28)	64 (50)
Total	77 (59)	55 (25)	58 (42)	63 (43)

*Interval—the time in days between the completion of STAMP I and the diagnosis of acute lung injury.

Pharmacokinetic Analysis

To evaluate the possibility that amifostine might alter the pharmacokinetics (PK) of BCNU, 17 patients randomized to placebo and 14 patients randomized to amifostine (all from the University of Colorado) underwent comprehensive PK blood sampling and analysis. Blood sampling, analytical analysis, and modeling were performed as described previously [15]. WinNonlin (Pharaight Corp, Mountain View, CA) was used for noncompartmental PK modeling. Derived PK parameters for the 2 groups were compared by using the Fisher exact test.

RESULTS

Patient characteristics are shown in Table 1 (placebo versus amifostine). These data demonstrate that there was balance by disease stage and patient age among the 3 participating sites. Seventy (90%) of the 78 patients who participated in this trial were treated for primary breast cancer. The mean age of patients in this study was 46 years. The mean age of patients who developed acute lung injury was identical to that of those who did not (46 years). In addition, Table 1 summarizes the intervals between STAMP I treatment and the diagnosis of acute lung injury by cohort. The mean time from treatment to diagnosis was 63 days and did not differ between the placebo and amifostine-treated groups.

Amifostine can produce hypotension when used with conventional chemotherapy, as does BCNU in the STAMP I regimen. There was concern that the nature of this side effect and the dosing proposed might result in unacceptable hypotension or delays or omissions of BCNU dosing. In fact, none of these problems occurred, and all treated patients received full doses of study drug and BCNU at the prescribed times. It should be noted that all patients were receiving hyperhydration (250 mL/m^2/h) during the period these drugs were administered, as dictated by the treatment protocol.

Table 2 shows the incidence of acute lung injury for amifostine-treated and placebo groups. As can be seen, no significant differences were noted between

Table 2. *Frequency of Diagnosis of Acute Lung Injury by Center and Treatment Group*

Amifostine	Colorado	Stanford	Karmanos	Total
Total	34	32	12	78
ALI, n (%)	20 (59)	27 (84)	8 (67)	55 (71)
Amifostine	16	15	8	39
ALI, n (%)	10 (63)	14 (93)	6 (75)	30 (77)
Placebo	18	17	4	39
ALI, n (%)	10 (56)	13 (76)	2 (50)	25 (64)

ALI indicates acute lung injury.

groups. In fact, the absolute incidence of acute lung injury in the amifostine-treated group (77%) exceeded that of the placebo group (64%; $P = .46$). The overall incidence of diagnosed acute lung injury was 71%. The incidences of acute lung injury from patients treated at Stanford (27/32; 84%), Karmanos (8/12; 67%), and Colorado (20/34; 59%) all exceeded historical data on the incidence of acute lung injury produced by the STAMP I regimen.

Table 3 shows the PK data from the treatment and placebo groups. No significant differences between the BCNU area under the plasma disappearance curve (AUC) and maximal plasma concentration were seen. Although amifostine was administered after cyclophosphamide and 95% of the cisplatin had been administered, we evaluated the possibility that that there were differences in cyclophosphamide or cisplatin PK between the 2 groups. No such differences were observed (data not shown).

If amifostine produced a partial protective effect, it would be reasonable to hypothesize that the intervals from BCNU treatment to the diagnosis of acute lung injury might be longer for patients receiving amifostine (63 days) than for placebo (63 days), but they were identical. All surviving patients were observed for 6 months for acute lung injury. The median ± SD of the time to development of acute lung injury was 63 ± 43 days, strongly suggesting that it is likely that <5% of acute lung injury diagnoses were missed because of this design feature.

Seventy of the 78 patients evaluable in this study were treated in the adjuvant setting. This factor, and the previously described study design, do not permit analysis of tumor outcome between the 2 groups. Finally, 1 of the 78 patients died as a result of acute lung injury. This patient received amifostine. One other patient died of nonpulmonary regimen-related toxicity, for a total toxicity mortality rate of 3%.

DISCUSSION

Under the conditions used in this study, the administration of 2 conventional doses of amifostine before the BCNU used in the STAMP I regimen did not protect against acute lung injury. This was the first trial designed to test this tissue-protective activity. Although numerous other randomized and phase II trials have suggested protection against peripheral neuropathy, ototoxicity, marrow toxicity, gastrointestinal mucositis, and other tissues by both chemotherapy and radiation, no such benefit was observed in this study. In fact, the study was stopped after a planned interim analysis suggested the high probability that the trial would be negative.

The STAMP I regimen was used primarily to treat patients with breast cancer. A recent randomized trial, presented in abstract form, questioned the benefit of this treatment in a study containing older patients than those reported here [16]. Although this result may lessen the clinical importance of STAMP I, BCNU is used routinely for hematopoietic cell–supported treatment of lymphoma in both the cyclophosphamide, BCNU, and VP-16 [17] and BCNU, etoposide, cytarabine, and melphalan [18] regimens, for example. Thus, the toxicity question asked here has ongoing and significant clinical relevance. The trial design did not include relapse or survival as end points, because considerable data exist that show that amifostine does not aversely affect these parameters [19] and because the predominance of primary breast cancer included in this trial would require prolonged follow-up and, likely, larger patient numbers for meaningful analysis.

Several factors could be responsible for our failure to observe lung protection: (1) failure to administer amifostine in an optimal manner, (2) lack of correlation between BCNU dose and pulmonary injury produced by STAMP I, (3) failure of amifostine to neutralize BCNU or its metabolites in lung tissue for chemical or pharmacodynamic reasons, (4) failure to diagnose acute lung injury uniformly between the 2 patient groups, or (5) overdiagnosis of acute lung injury with sufficient frequency to obscure any protective effect of amifostine.

Amifostine has been shown to be distributed to a variety of normal tissues, including the lung, and hydrolyzed rapidly to its active metabolite WR-1065 [7]. Wasserman et al. [20] showed that amifostine pro-

Table 3. *Derived Pharmacokinetic Parameters for BCNU by Treatment Group**

Variable	AUC (μg-min/mL)	$T_{1/2elim}$ (min)	C_{max} (μg/mL)
Amifostine, mean (SD)	415 (74)	15 (8)	3.4 (0.6)
Placebo, mean (SD)	456 (150)	18 (8)	3.7 (1.3)

AUC indicates area under the plasma disappearance curve; $T_{1/2elim}$, elimination half-life; C_{max}, maximal plasma concentration.

*A total of 15 patients receiving amifostine and 15 patients receiving placebo from whom total pharmacokinetic data were available were included in this analysis. All these patients were treated at the University of Colorado.

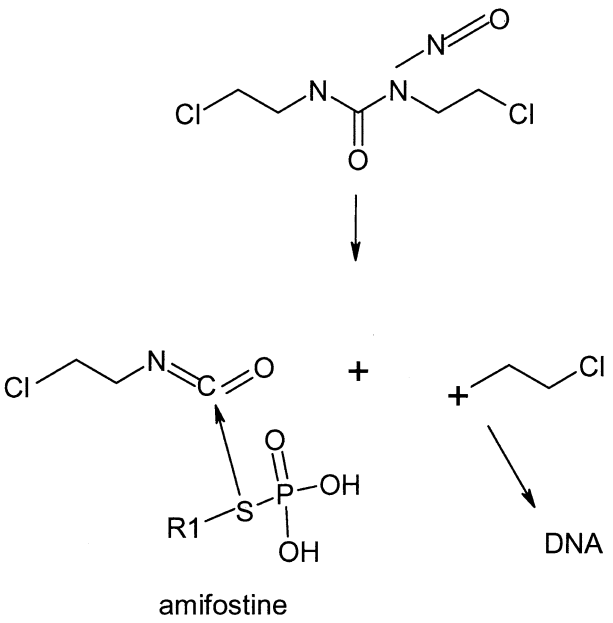

Figure 1. R1 = 2-ethyl-[3 aminopropyl aminoethyl].

vided significant cytoprotection to ex vivo cultured hematopoietic progenitors treated with BCNU, demonstrating that chemically, the concept of amifostine providing cytoprotection from BCNU was reasonable.

Amifostine is typically administered in a dose between 740 and 910 mg/m^2 given 30 minutes before chemotherapy. This trial was initiated in a period when the 740 mg/m^2 dose was recommended, so it was used for this study. This dose produces an average plasma amifostine AUC of 1100 μmol-min/L, whereas the BCNU AUC from STAMP I is typically 2400 μmol-min/L. Because the active metabolite of amifostine, WR-1065 [7], is hypothesized to neutralize reactive intermediates by 1:1 covalent binding, 2 doses of amifostine were administered in this study to ensure that approximately equal molar equivalents BCNU and amifostine were given to the study subjects. Hydrolysis of BCNU, however, produces a highly reactive 2-chloroethyl-carbonium ion, as well as 2-chloroethyl isocyanate, both of which could react with WR-1065 (Figure 1). Although it is generally suggested that glutathione reacts substantially with the isocyanate but not the carbonium ion [21], it is possible that the dose of amifostine was insufficient to neutralize the drug in lung tissue. Further, carbonium ions, because of their instability, cannot circulate after formation, but rather will react with the closest molecule possessing a negative charge. Thus, they might produce their effects primarily intracellularly. The isocyanate, however, has a half-life of a few minutes in the circulation and thus might primarily act at the cell membrane if the primary site of BCNU hydrolysis is intravascular.

Others have suggested that the conjugation of 2-chloroethyl isocyanate with glutathione is reversible and that the conjugate, in effect, may stabilize the isocyanate and enhance its toxicity [22]. The chemical activity of WR-1065 mimics the activity of glutathione. Because WR-1065 exists primarily in the intracellular space, it is possible that WR-1065, the active metabolite of amifostine, exists in a low concentration at the cell membrane, where the isocyanate might produce its toxic effects. It is reasonable to hypothesize that 2-chloroethyl isocyanate might be the primary cause of BCNU-induced lung injury, because its close chemical cousin, methyl isocyanate, was the cause of extensive pulmonary injury after a well-publicized industrial accident in Bhopal, India (ICMR Report [1986] Health effects of the Bhopal gas tragedy. Indian Council of Medical Research, New Delhi) [23]. It seems likely that attempts to substantially increase the dose of amifostine to compensate for these factors might produce unacceptable hypotension, the well-recognized acute toxicity of amifostine.

BCNU is widely recognized as a pulmonary toxin [24]. When administered in hematopoietic cell transplant doses, a syndrome of acute lung injury is frequently seen [9,12,25,26]. The injury manifests as dyspnea, cough, and fever or as asymptomatic abnormalities in pulmonary function tests. The chest radiograph is most often normal. This injury most frequently occurs between 1 and 3 months, and rarely later than 6 months, after BCNU treatment. The injury is highly corticosteroid responsive if diagnosed early, and the natural history of the injury after STAMP I has been described in detail. Some reports [27], but not others [11], have described a pharmacodynamic relationship between the BCNU AUC and pulmonary drug injury when the STAMP I regimen is used. Because the incidence of lung injury produced by cyclophosphamide or cisplatin when used as single agents in comparable doses is small, it seems unlikely that they cause the lung injury produced by STAMP I. Further, comprehensive analyses of the PK of STAMP I suggest that, in the same patients in whom pharmacodynamic correlations between BCNU and lung injury occur, there is no correlation with the PK of either cyclophosphamide or cisplatin [27]. These data, taken together, suggest that the administration of amifostine before BCNU represents a reasonable pharmacologically designed attempt to provide lung cytoprotection from STAMP I.

Finally, the STAMP I regimen and its acute lung injury syndrome are well recognized and described. Patients treated with STAMP I were carefully instructed to report dyspnea, cough, or fever to their personal oncologists or transplant center physicians at once. They were provided with written instructions to transmit to physicians caring for them at home about diagnosis and treatment of the lung injury. The inci-

dence of acute lung injury reported for both the amifostine- and placebo-treated groups in this trial varied somewhat among the 3 participating centers. Most notably, however, the incidence of clinically diagnosed acute lung injury was higher in all 3 centers than the frequency anticipated from historical data. To evaluate the possibility that overdiagnosis of acute lung injury might have obscured the analysis, we inspected the subset of 34 patients treated at the University of Colorado, whose frequency of diagnosis of acute lung injury was the lowest and most comparable to historical data (59%). In this subgroup, the frequencies of acute lung injury in patients treated with amifostine (10/16; 63%) and placebo (10/18; 56%) were comparable ($P = .74$), suggesting that neither overdiagnosis nor amifostine use could explain the outcome reported here.

In conclusion, we were unable to detect any reduction in acute lung injury associated with the STAMP I regimen as a result of amifostine administration. This result should be viewed as specifically relating to the BCNU used in STAMP I, and it does not reflect on the ability of amifostine to provide cytoprotection with other agents or regimens. We do not believe, however, that altering the dose or schedule of amifostine is likely to enhance amifostine cytoprotection from BCNU-associated acute lung injury. The high frequency of acute lung injury observed in this study reinforces this view. Further studies of amifostine/BCNU combinations should be conducted only after careful attention to the results presented here.

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